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A suitable model for the utilization of *Duddingtonia flagrans* fungus in small-flock-size sheep farms

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ABSTRACT

Effective alternatives to anthelmintic treatment of nematode parasite infections of sheep are required because of the high prevalence of drug resistance. Within this context, the nematode-trapping fungus *Duddingtonia flagrans* has become a valuable component of various integrated control strategies. Toward this objective, a small quantity of lyophilized *D. flagrans* chlamydospores (10^6 spores per animal) was administered to sheep in a one-year plot study. Animals grazing on native pasture were divided into two homogeneous groups and were kept in 1-ha paddocks in the southern region of Brazil. The oral administration of chlamydospores led to a significant reduction ($p < 0.05$) in the number of nematode eggs per gram of feces and in the larval availability on herbage (difference of 37.6%) in comparison to the control group. Control animals needed to be dewormed three times during the experiment, whereas the fungus-treated animals maintained a low parasite load, independent of seasonal variation. Although *D. flagrans* cannot serve as a panacea for nematode parasite control of livestock, it represents a significant advance toward rationalizing the use of endoparasitic drugs in small animals.

1. Introduction

Sheep husbandry is a worldwide activity that can be profitable in the medium term and in which success is directly related to the variable costs. A great part of these costs are composed by input costs, which can reach 30% of the total operating cost on farms in southern Brazil (Viana and Silveira, 2009). Over the years, nematode parasites of sheep and goats, which also pose a great limitation to the profitability of small ruminants farming, have been effectively controlled by the use of anthelmintics. In addition to anthelmintics being costly and environmentally unfriendly, the frequent and indiscriminate use of these substances has led to the development of resistance in worm populations (Chandrawathani et al., 2003). In parallel, alternative control approaches have been developed to supplement chemoprophylaxis.

The Famacha method, which is based on the identification of resistant and resilient animals within the flock, selecting animals that do not require drug treatment (Molento et al., 2004), and pasture rotation management (Larsson et al., 2007) are useful natural alternatives with proven efficacy. Moreover, biological control using nematophagous fungi has emerged and gained popularity in the control of gastrointestinal parasites of herbivores (Waller et al., 2001; Knox and Faedo, 2001). The administration of chlamydospores of the predacious fungus *Duddingtonia flagrans* in animal food leads to a reduction in the pre-parasitic stages of gastrointestinal nematodes in the feces (Mendonza de Gives et al., 1998).

Among the nematode parasites of sheep in southern Brazil, *Haemonchus contortus* represents the major problem during the summer months. *Trichostrongylus* spp. and *Ostertagia* spp. assume dominance during the winter and spring, causing widespread clinical disease and productivity losses (Echevarria et al., 1996). As a result, efficient control of worm infections should be done continuously throughout the year. Daily feeding of chlamydospores is even more important than the uniform intake of fungal spores per sheep because the behavioral habit of feeding near dung piles provides chlamydospore ingestion directly from the environment (Santurio et al., 2009).

The purpose of our field trial was to study the efficacy of a low amount of *D. flagrans* chlamydospores fed to sheep over a one-year period and to report data on fungal elimination, host parasite load, pasture contamination level and weather fluctuations during the different seasons.

2. Material and methods

2.1. Fungal cultures

The *D. flagrans* isolate, strain ARSEF 5701 (Collection of Entomopathogenic Fungal Cultures), was used. Fungal chlamydospores

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were obtained according to the method described by Santurio et al. (2009).

2.2. Experimental design

The experiment was conducted on a rural property in the municipality of Itaara, Rio Grande do Sul state, southern Brazil, at a latitude of 29°36′35″S and longitude of 53°45′53″W, altitude of 425 m, from May 2009 to April 2010. The area is characterized by a humid subtropical climate, with an average yearly temperature and precipitation of 18.5 °C and 1700 mm, respectively. 20 animals aging six-months, female sheep were divided into two homogeneous groups of 10 animals each. Each group was kept in individual 1-ha paddocks with native pasture. Between May and September 2009, animals were allowed to graze ryegrass pasture for 2 h/day in different paddocks, and between December and April, animals grazed oat pasture. Notably, the animals from different groups were not allowed to graze at the same paddock. Moreover, animals were supplemented with alfalfa from November to December.

2.3. Treatment

Each sheep in the treated group was fed 10 g of ground corn, containing an average of 10^6 lyophilized *D. flagrans* chlamydospores, daily. Control animals received the same amount of feed. Animals were submitted to a 20-day adaptation period before the beginning of the experiment, during which time all of the animals received a single dose of oxfenbendazole (2.5 mg/kg BW) *per os*.

To limit pasture contamination and subclinical disease, an oral dose of levamisole (15 mg/kg BW) was administered to the animals whenever the median egg count of the group reached 500 eggs per gram of feces (EPG). We chose to use the median EPG because the mean EPG can be easily influenced by outliers.

2.4. Fecal examination

Samples of fresh feces were collected once per month directly from the rectum and were transported in refrigerated plastic bags to determine the EPG, according to the method of Gordon and Whitlock (1939) and modified by Lima (1989), and to determine the number of chlamydospores per gram of feces (CPG), according to the method of Da Silva et al. (2009).

Coprocultures were established together with EPG counts; 20 g of feces were mixed with autoclaved wood shavings and kept moist at a controlled temperature (26 °C) for 7 days to obtain trichostrongylid larvae. Larvae were identified to the genus level as described by Ueno and Gonçalves (1998).

2.5. Pasture larval count

Every 30 days, herbage samples were collected in each paddock of both the treated and control groups, in a zigzag pattern from several and alternated points, according to Amarante et al. (1996). Herbage samples were always collected at 9 a.m. Then, a 500-g herbage sample was weighed out, and parasitic nematode larvae were recovered following the procedure of Lima (1989). The samples were incubated in a drying oven at 100 °C for 3 days to determine the dry matter content. Data were transformed into larvae per kg of dry matter.

2.6. Meteorological data

Climate data including the average temperature, relative humidity and monthly rainfall were obtained from a local meteorological station.

2.7. Data analysis

The percentage reduction in the herbage larval population was estimated using the following formula: % reduction = XC − XT/XC × 100 (XT = mean number of larvae recovered from the treated group; XC = mean number of larvae recovered from the control group). The EPG data were analyzed by the Mann–Whitney test ($p < 0.05$).

3. Results

Statistically significant differences ($p < 0.05$) in EPG counts were observed in the months of June and October of 2009 (Fig. 1A). In fact, control animals had to be dewormed three times during the experiment because the median EPG of the group reached 500 (in June and October of 2009, during which time six animals had an EPG above 500, and in March of 2010, during which time seven animals had a high EPG). The median EPG of the control group at the end of the experiment was 200, with a range of 0–750. Conversely, animals receiving 10^6 *D. flagrans* chlamydospores daily did not require treatment (EPG < 500), and notwithstanding, the EPG values were lower than those of the control group in general (median EPG of 100; EPG range 50–450). The mean CPG counts of the fungus-treated group remained constant during the entire study period (mean CPG, 537; CPG range, 430–670; Fig. 1B). Meanwhile, no chlamydospores were found in the control group.

The most prevalent Trichostrongylidae genera identified in coprocultures were as follows, in decreasing order: *Haemonchus*, *Trichostrongylus*, *Ostertagia* and *Oesophagostomum* (Fig. 2). *Haemonchus* spp. and *Trichostrongylus* spp. always contributed the greatest number of eggs to the total egg output in the warm and cold seasons, respectively. No differences were observed between the groups regarding the predated nematode species.

The pasture larval load varied according to the season of the year, being lower in May through October of 2009 and higher in November of 2009 through April of 2010 (Fig. 3). The mean number of larvae recovered from paddocks increased with the elevation of the temperature, with the monthly means of the control group (mean larval count of 13,000; range 4800–22,000) always being greater than those of the treated group (mean larval count of 8100; range 3300–10,300). The mean trapping percentage of the lyophilized chlamydospores at the end of the study was more than 37%, as compared to the control group.

4. Discussion

Many studies have attempted to determine the optimum dose of *D. flagrans* chlamydospores offered *per os* to the animals. Results from plot studies with sheep nematodes have been variable. Faedo et al. (1998) reported a 43% reduction in grass infectivity with 1–1.25 × 10^5 chlamydospores/kg BW on *H. contortus*, whereas Faedo et al. (2000), with 10^6 spores/kg BW, found a higher reduction (80–90%) in *Teladorsagia/Trichostrongylus*. According to Githigia et al. (1997), the dose of 10^6 spores given to lambs infected with *Teladorsagia/Trichostrongylus* during a four-month grazing period reduced the pasture larval counts by 78% at the end of the experiment. Unsatisfactory results were obtained with 2.5 × 10^5 spores/kg BW, where the variability in the larval count reduction was attributed to meteorological factors and to sub-optimal *Duddingtonia* spore dosage (Chartier and Pors, 2003). Because the production of *D. flagrans* chlamydospores is limited to few research centers and universities and often requires techniques that involve culture media (e.g., potato dextrose agar) and industrial equipment (e.g., autoclave), we decided to use a 50-fold lower dosage (10^6 spores per animal) in our experiment, relative to the dosage of 10^6

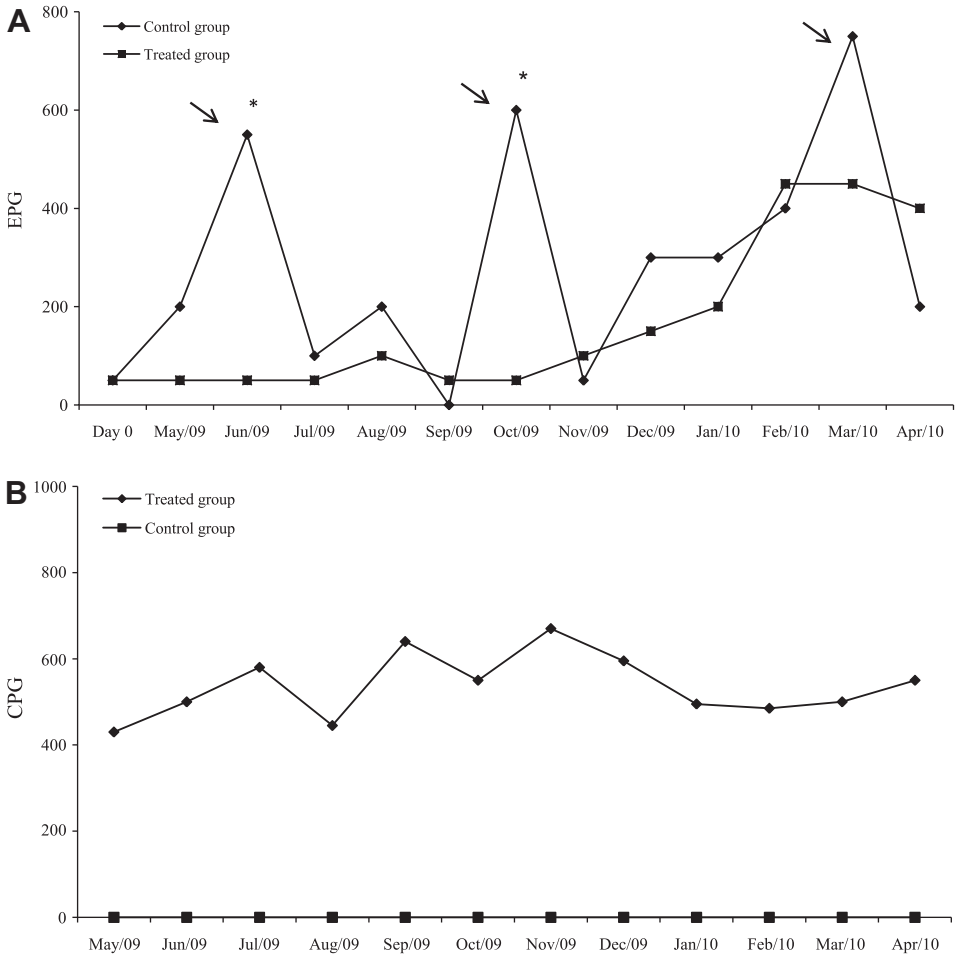

Fig. 1. (A) Monthly medians of number of eggs per gram of feces (EPG) and (B) monthly means of the number of chlamydospores per gram of feces (CPG) of fungus-treated sheep and control collected from May of 2009 to April of 2010. Asterisks indicate statistically significant differences between groups (Mann–Whitney test; $p < 0.05$). Arrows denote therapeutic treatment of control group (levamisole 15 mg/kg BW).

spores/kg BW used in field studies elsewhere (Faedo et al., 2000; Githigia et al., 1997; Dimander et al., 2003), and an average weight of 50 kg per sheep. The results obtained in our study were outstanding and significantly better than current worm control technologies, such as the use of anthelmintics. Although both the control and fungus-treated groups were dewormed at the beginning of the experiment, the control group required three additional dewormings throughout the one-year trial, whereas the median EPG counts of the treated group remained below 500, indicating no need for drug treatment. The trapping efficacy of the lyophilized *D. flagrans* chlamydospores previously reported *in vitro* (Santurio et al., 2009) was observed here in a field study, corroborating the feasibility of this production technique.

It has now largely been acknowledged that effective parasite control cannot be persistently achieved using a single method. Although the use of anthelmintics could play a crucial role in such integrated control, their use should be minimized as much as possible (Molento et al., 2004; Sanyal et al., 2004). However, the inherent susceptibility of *D. flagrans* to benzimidazole fungicides should be taken into consideration when the administration of a benzimidazole anthelmintic is to be integrated with biological control in sustainable worm management (Sanyal et al., 2004).

The results showed concomitant decreases in EPG counts and in the levels of pasture infestation in both groups in July, when the average monthly temperature hovered around 10 °C (Fig. 4). However, neither the action of the nematophagous fungus in the treated group nor the anthelmintic use in the control group were capable of eliminate all of the parasites. According to Echevarria et al. (1996), benzimidazole, levamisole and combination products have virtually reached their limit of chemotherapeutic usefulness in southern Brazil, with resistance to these groups being higher there than in any other sheep production region in the world. Together with the low temperatures, drought between the months of May and August of 2009 led to lower larval development and consequently less larval ingestion. These conditions also contributed to a decrease in infection with *Haemonchus* spp., which is benefited by rainy periods, whereas these conditions favor the development of *Trichostrongylus* spp. (Ramos et al., 2004). No differences in the parasite species were observed in the coprocultures of the treated and control groups. Notably, *D. flagrans* is often considered to be non- or lowly discriminant with respect to digestive nematode prey species (Paraud et al., 2006).

In addition to increasing temperatures, the highest mean rainfall, recorded in November, is likely to have aided in dislodging and dispersing helminth larvae in pastures, what, therefore, could have impaired the ability of *D. flagrans* to avoid the larvae spreading from feces to the grass (Dimander et al., 2003) because the action of the fungal adhesive nets occurs in the dung (Mendonza de Gives et al., 1998). This situation was not observed in our study because a reduction of 53.7% in the number of larvae on herbage was recorded in the fungus-treated group in November (Fig. 3). Similar results were found by Jobim et al. (2008), who reported similar

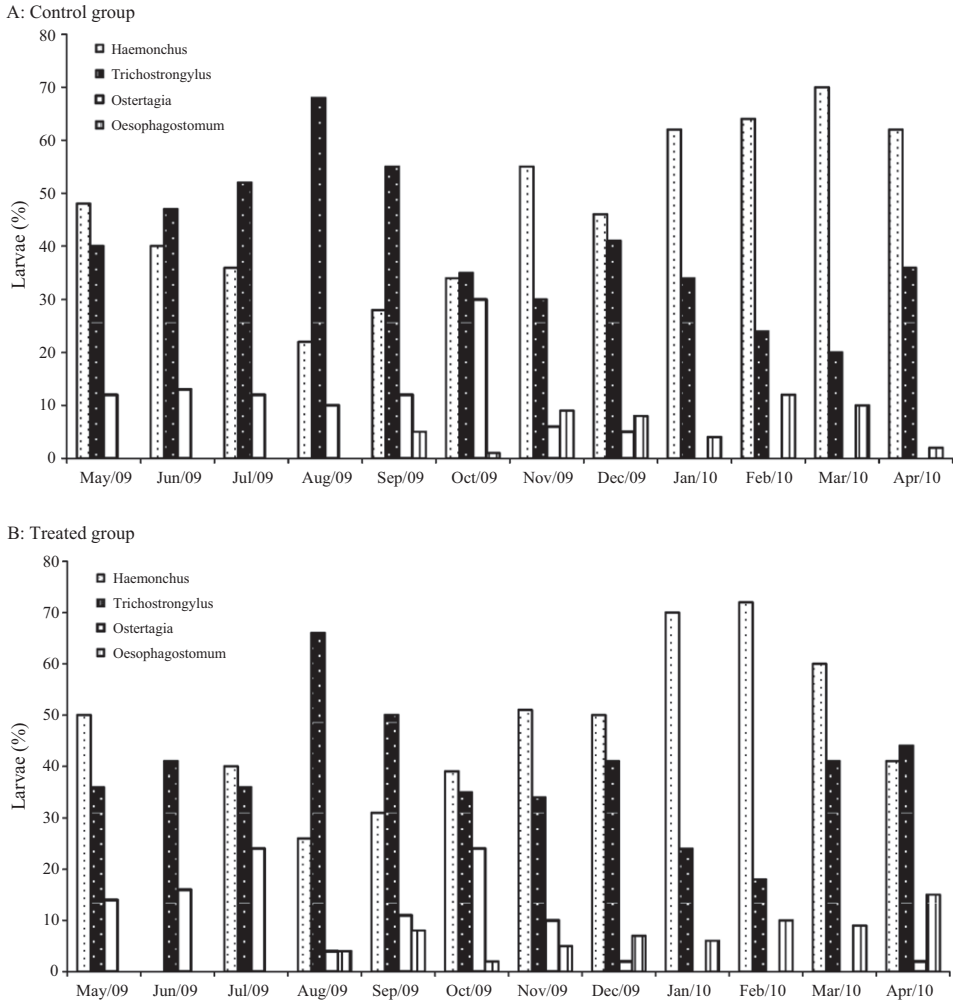

Fig. 2. Percentage of Trichostrongylidae larvae recovered from coprocultures of fungus-treated sheep (A) and control group (B), collected from May of 2009 to April of 2010.

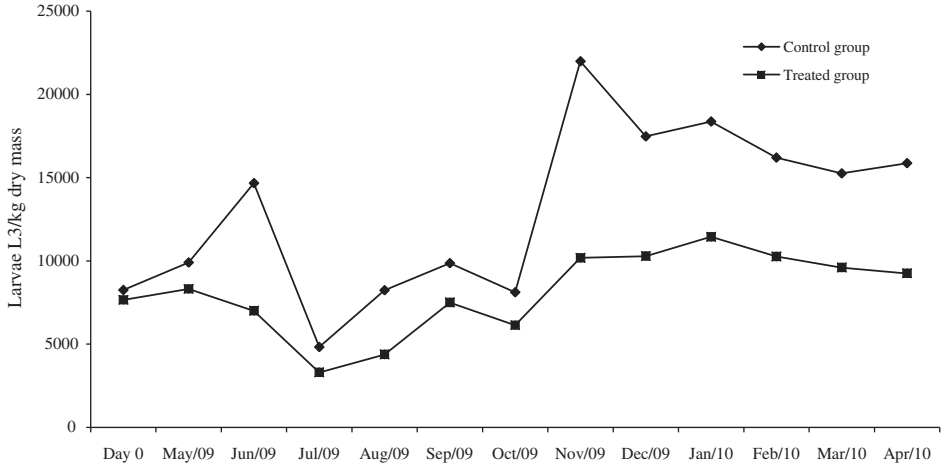

Fig. 3. Monthly counts of the number of infective nematode larvae per kilogram of dry matter recovered from pastures of fungus-treated sheep and control, collected from May of 2009 to April of 2010.

pasture larvae levels, in drought and rainy periods, in paddocks with cattle treated with *D. flagrans*. The monthly CPG counts remained practically constant throughout the experiment, in agreement with the results of Ojeda-Robertos et al. (2008), who reported that the number of CPG is clearly related to the dose of chlamydospores offered *per os*.

The results of this experiment clearly demonstrate that a small quantity of *D. flagrans* chlamydospores, whether used in

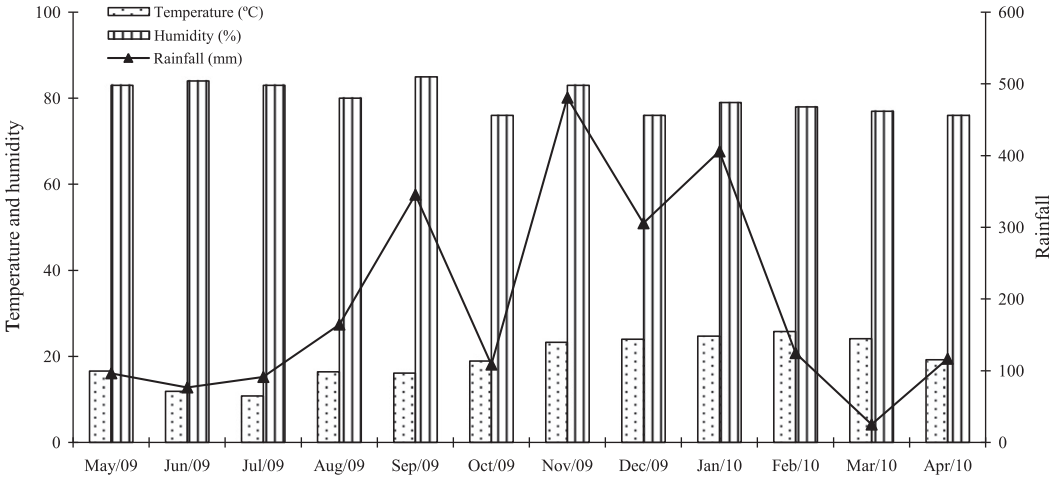

Fig. 4. Averages of temperature, air relative humidity and rainfall recorded between May of 2009 and April of 2010.

combination with other worm control strategies, is a suitable alternative for small-flock-size farms. Although nematophagous fungi might not completely eliminate the use of anthelmintics in the long term, the formulation containing lyophilized *D. flagrans* chlamydospores could be applicable in the sheep husbandry, with the aim of reducing drug usage and prolonging the usefulness of drugs for the treatment of parasite infections.

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